BBA Clinical 5 (2016) 159–165

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: www.elsevier.com/locate/bbaclin

# *TCF7L2* polymorphism is associated with low nitric oxide release, endothelial dysfunction and enhanced inflammatory response after myocardial infarction☆

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ARTICLE INFO

*Article history:*
Received 5 January 2016
Received in revised form 23 March 2016
Accepted 24 March 2016
Available online 2 April 2016

*Keywords:*
TCF7L2 polymorphism
Endothelial function
Myocardial infarction
Mortality

ABSTRACT

*Backgound:* The favorable effects of insulin during myocardial infarction (MI) remain unclear due to the divergence between mechanistic studies and clinical trials of exogenous insulin administration. The rs7903146 polymorphism of the *transcription factor 7-like 2* (TCF7L2) gene is associated with attenuated insulin secretion.
*Methods:* In non-diabetic patients with ST-elevation MI (STEMI), using such a model of genetically determined down-regulation of endogenous insulin secretion we investigated the change in plasma insulin, C-peptide, interleukin-2 (IL-2), C-reactive protein (CRP), and nitric oxide (NOx) levels between admission (D1) and the fifth day after MI (D5). Coronary angiography and flow-mediated dilation (FMD) were performed at admission and 30 days after MI, respectively. Homeostasis Model Assessment estimated insulin secretion (HOMA2%β) and insulin sensitivity (HOMA2%S).
*Results:* Although glycemia did not differ between genotypes, carriers of the T-allele had lower HOMA2%β and higher HOMA2%S at both D1 and D5. As compared with non-carriers, T-allele carriers had higher plasma IL-2 and CRP at D5, higher intracoronary thrombus grade, lower FMD and NOx change between D1 and D5 and higher 30-day mortality.
*Conclusion:* In non-diabetic STEMI patients, the rs7903146 *TCF7L2* gene polymorphism is associated with lower insulin secretion, worse endothelial function, higher coronary thrombotic burden, and higher short-term mortality.
*General significance:* During the acute phase of MI, a lower capacity of insulin secretion may influence clinical outcome.


## 1. Introduction

Abundant data ranging from cell models to mechanistic studies in human subjects have indicated that insulin may offer a wide array of potentially positive effects on the cardiovascular system. For example, insulin has been shown to improve endothelial function [1], reduce inflammatory response [2], increase myocardial blood flow [3], and attenuate both platelet aggregation [4] and pro-thrombotic factors [5]. These effects are particularly interesting in the setting of myocardial infarction (MI), which is when these mechanisms are playing pivotal roles in determining clinical outcome. Thus, it is conceivable that β-cell capacity may be intrinsically involved in the adaptive response to MI.

Recently, a variant of the *transcription factor 7-like 2* (TCF7L2) gene, the rs7903146 (IVS3C>T), was associated with development of type 2 diabetes (T2D) [6,7]. It is a rather common single nucleotide polymorphism (SNP) that is associated with posttranscriptional impairment in insulin secretion [8]but has no effect on insulin sensitivity [9,10]. In this context, we hypothesize that carriers and non-carriers of the rs7903146 mutant allele portray different endogenous insulin output during the acute phase of MI and, if so, they will demonstrate distinct adaptive responses to MI. Hence, we investigated in nondiabetic individuals with MI the association between rs7903146 genotype

☆ R.C., F.A.M and L.S.F.C researched data, conducted data analyses and prepared the manuscript. M.D. performed genotyping and edited the manuscript. S.N.S. performed FMD experiments. A.P.R.C researched data regarding dietary evaluation. J.A. conducted data analyses regarding CMRI. V.N.F., F.A.R.N., J.C.Q.S. and A.C.S reviewed the manuscript. A.C.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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http://dx.doi.org/10.1016/j.bbacli.2016.03.010

TCF7L2 and outcome-related mechanisms. In addition, we explored its association with the recurrence of cardiovascular events.

## 2. Research design and methods

### 2.1. Study patients

Patients are participants in the *Brasilia Heart Study* (ClinicalTrials.gov Identifier: NCT02062554), an ongoing prospective cohort of consecutive ST-elevation MI (STEMI) patients that were enrolled between May 2006 and October 2011 (flow diagram in Fig. 1). Inclusion criteria are as follows: (i) <24 h after the onset of MI symptoms, (ii) ST-segment elevation of a least 1 mm (frontal plane) or 2 mm (horizontal plane) in two contiguous leads, and (iii) myocardial necrosis, as evidenced by an increase to at least one value above the 99th percentile above the reference limit of CK-MB (25 U/L) and troponin I (0.04 ng/mL) followed by a decline of both. Individuals with a previous diagnosis of diabetes, use of hypoglycemiants, or glycosylated hemoglobin (HbA1c) ≥6.5% (48 mmol/mol) were excluded from the study. There was no interference with patient treatment, which was decided by the assistant physician following a hospital protocol based on the current ACC/AHA guidelines for STEMI. The physicians involved in the treatment of the patients were blind to all analyses performed in the study. The local Ethics Committee approved the study and all participants signed an informed consent. The authors vouch for the integrity of the data and all analyses. All authors have read and agree to the manuscript as written.

### 2.2. Biochemical analysis

Blood samples were obtained at D1, *i.e.* <24-h from MI onset, and at day five following MI (D5). Samples were centrifuged for 10 min at 3500 rpm. Plasma was aliquoted for storage at −80 °C. The following methods were used for analyses of plasma samples: glucose (GOD-PAP, Roche Diagnostics, Mannheim, Germany), total cholesterol (CHOD-PAP, Roche Diagnostics, Mannheim, Germany), triglycerides (TG) (GPO-PAP, Roche Diagnostics, Mannheim, Germany), high-density lipoprotein cholesterol (HDL-C) (Roche Diagnostics, Mannheim, Germany), C-reactive protein (CRP) (Cardiophase, Dade Behring, Marburg, Germany), 8-isoprostane (EIA kit, Cayman Chemical Company, Ann Arbor, MI, USA), interleukin-2 (IL-2) (Fluorokine® MAP Human IL-2 Kit, R&D Systems, Minneapolis, MN, USA), and HbA1c (Variant II, Bio-Rad Laboratories, Hercules, CA, USA). Plasma levels of nitrite and nitrate (NOx) were measured by an NO chemiluminescence analyzer (model NOA, Sievers Instruments, Boulder, CO) after reduction with acidic vanadium (III) chloride. Plasma insulin and C-peptide concentrations were determined by electrochemiluminescence (Roche Diagnostics, Mannheim, USA) and by imunoquimioluminescence (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA, USA), respectively. The Homeostasis Model Assessment version 2 (HOMA2) was used to estimate β-cell function (HOMA2%β) and insulin sensitivity (HOMA2%S), which were both calculated with HOMA2 calculator version 2.2. [11] We used fasting plasma insulin levels to compute HOMA2%S and plasma C-peptide to compute HOMA2%β. The accuracy of HOMA2 index during STEMI was previously validated in STEMI patients by euglycemic hyperinsulinemic clamp in our laboratory. [12]

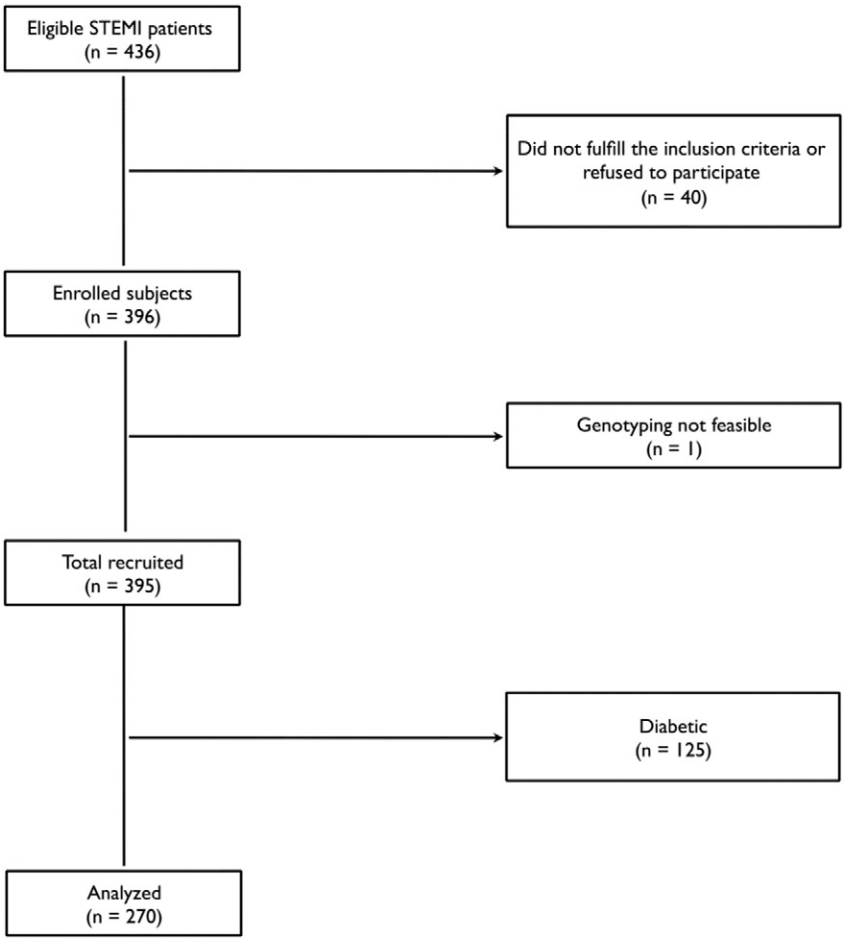


**Fig. 1.** Flow-chart of patient enrollment.

### 2.3. Genotyping

DNA was extracted from buffy coat using QIAamp DNA Blood Mini Kit (Quiagen, GmBH Hilden) and stored at −20 °C. Genotyping was determined by real-time amplification refractory mutation system using allele-specific primers. Two forward primers were designed with a mismatch in their last 3′ nucleotide in such a way that each was specific for the two variants of the polymorphism (NCBI RefSNP: 7903146): variant C GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC; and variant T GAACAA TTAGAGAGCTAAGCACTTTTTAGAGAT. A common reverse primer without mismatches, AGATGAAATGTAGCAGTGAAGTGC, was designed downstream of the polymorphic site. Two parallel real-time polymerase chain reactions were conducted with the combined use of allele-specific forward primers and a common reverse primer, in duplicates. The amplification program was: DNA polymerase activation at 95 °C for 10 min, followed by 35 repeated cycles of denaturation at 95 °C for 15 s and annealing-extension at 60 °C for 1 min. Amplification specificity was tested by melt curve analysis, which was performed by heating the samples from 60 °C to 95 °C at increments of 0.3 °C while recording the fluorescence signal decay. Genotyping reactions were performed in batches of 20 samples. Gene amplification was carried out using a *SYBR® green* assay (Maxima SYBR® Green qPCR Master Mix 2X, Fermentas, Glen Burnie, MD, USA) in a Step One™ Real-Time qPCR System (Applied Biosystems, Foster City, CA, USA).

### 2.4. Coronary angiography

Coronary angiography was systematically performed on all patients enrolled to assess coronary thrombotic burden, degree of reperfusion, and severity of atherosclerotic disease. Thrombotic burden was estimated at the culprit coronary artery according to the Thrombolysis In Myocardial Infarction (TIMI) Thrombus Grade Scale as follows: (i) Grade 0—no angiographic evidence of thrombus is present; (ii) Grade 1—possible thrombus, with reduced contrast density, haziness, irregular lesion contour, or a smooth convex at the site of total occlusion suggestive but not diagnostic of thrombus; (iii) Grade 2—definite thrombus, with greatest dimensions less than half of vessel diameter; (iv) Grade 3—definite thrombus but with greatest dimensions less than half but greater than two vessel diameters; (v) Grade 4—definite thrombus greater than two vessel diameters; and (vi) Grade 5—total occlusion [13]. The Gensini score estimated coronary disease severity. Coronary flow in the culprit artery was measured using TIMI flow grade and Myocardial Blush Grade (MBG), and was assessed after angioplasty in patients who underwent primary percutaneous coronary intervention (PCI) or at the earliest possible angiogram performed after thrombolysis.

### 2.5. Brachial artery reactivity

Brachial artery measurements were performed using a high-resolution ultrasound (IE33 and 3–9 MHz linear transducer; Philips Medical Systems, Bothell, Washington, USA) after over-night fasting 30 days after admission (D30) and vasoactive medications were withdrawn 24 h before assessment. After 10 min of rest in a quiet room with the temperature controlled around 22 °C, the brachial artery was located above the elbow, and a longitudinal image of 6 to 8 cm was taken as the resting scan. A blood pressure cuff was placed on the forearm and inflated to 50 mm Hg above the systolic blood pressure for 5 min. The cuff was deflated, and the flow-mediated dilation (FMD) scan was obtained for 2 min. The percentage change in diameter for FMD was calculated in relation to the respective baseline scans. The same experienced physician, who was blinded to patients' data, analyzed brachial artery reactivity. The intra-observer reproducibility was 95%.

### 2.6. Cardiac magnetic resonance imaging (CMRI)

CMRI studies were carried out using a MRI scanner with a 1.5-T (Signa CV/i, GE Medical Systems, Waukesha, WI) equipped with a gradient of high performance (gradient strength 40 mT/m; maximum slew rate 150 mT/m/s) and a four element phased array cardiac coil. Areas of MI were quantified on the gadolinium-based delayed enhancement myocardial images at D30 to quantify MI mass, left ventricle (LV) volumes, and ejection fraction. Areas of microvascular obstruction (MVO) were defined as subendocardial hypo-enhanced regions surrounded by hyperenhancement and were included as part of the core infarct. On cine-CMRI, left ventricle (LV) volumes and ejection fraction were measured by ReportCard software (GE Medical Systems, Waukesha, WI) by applying Simpson's method.

### 2.7. Statistical analysis

Normally distributed data is presented as mean ± standard deviation and skewed data is presented in the form of median (interquartile range). Log transformation was applied to skewed variables in order to use parametric tests. The change in values for the variables was calculated as the relative difference between D5 and D1. Analysis of covariance (ANCOVA) was used for FMD, NOx, glycaemia, HDL-C, LDL-C, TG, CRP, 8-isoprostane, IL-2, insulinemia, C peptide, HOMA2%S and HOMA2%β. ANCOVA was used to compare these variables between groups after analysis with histograms, normality plots and residual scatter plots that tested for linearity, normality and variance. Mortality in the first 30 days was investigated by chi-square and binary logistic regression. A $<0.05$ two-sided p-value was considered significant. SPSS software version 21.0 was used for all analyses.

## 3. Results

### 3.1. Baseline characteristics

A total of 118 patients were homozygous for wild-type C-allele, 23 were homozygous for the mutant T-allele and 129 were heterozygous. The Hardy–Weinberg equilibrium was evaluated using the $\chi^2$ test ($p = 0.0158$), in which the T-allele was considered dominant and the frequency of the recessive allele was 0.33. Carriers of the T-allele were grouped together in order to increase statistical power. As shown in Table 1, study participants did not differ in terms of demographic characteristics, except that a larger percentage of patients with the T-allele used beta-blocker during hospitalization. Small differences were found in plasma TG and HDL-C between carriers and non-carriers of the T-allele.

### 3.2. Glucose metabolism

As shown in Table 3, plasma insulin levels were lower in the T-allele carriers at both D1 and D5 as compared to patients with CC genotype. Plasma C-peptide level was similar at D1 across genotype groups but lower at D5 in T-allele carriers. Consequently, the latter group had a more intense decline of C-peptide levels between D1 and D5. Consistently, pancreatic β-cell function estimated by HOMA2%β was lower in the T-allele carriers at D1 and D5.

Insulin sensitivity measured by HOMA2%S was higher in T-allele carriers at D1 and D5 as well as the change of HOMA2%S between D1 and D5. Blood glucose was similar across the genotype groups at D1 and D5 indicating that the lower insulin output was balanced by the higher insulin sensitivity.

### 3.3. Oxidative stress and inflammatory response

As shown in Table 3, systemic inflammatory activity at D5 estimated by plasma CRP levels was higher in carriers of the T-allele than in their

**Table 1**
Characteristics of studied patients grouped by the presence of the T-allele.

| | TCF7L2 rs7903146 | | p |
|---|---|---|---|
| | CC | CT + TT | |
| n | 118 | 152 (129 CT + 23 TT) | |
| Male (%) | 75 | 79 | 0.39 |
| Age (years) | 61.9 ± 10.5 | 62.3 ± 12.7 | 0.77 |
| BMI (kg/m$^2$) | 25.5(4.8) | 25.5(5.1) | 0.87 |
| Waist circumference (cm) | 94.7 ± 8.9 | 95.2 ± 12.2 | 0.74 |
| Family history for CAD (%) | 42 | 41 | 0.88 |
| Hypertension (%) | 56 | 54 | 0.75 |
| Smoking habit (%) | 37 | 45 | 0.18 |
| HDL-C (mg/dL) | 39(17) | 38(13) | <0.001 |
| LDL-C (mg/dL) | 130 ± 39 | 118 ± 42 | 0.28 |
| TG (mg/dL) | 104(77) | 121(87) | <0.001 |
| CK-MB Peak (UI/ml) | 168(231) | 155(202) | 0.54 |
| Creatinine clearance | 72 ± 20 | 70 ± 22 | 0.52 |
| Sedentarity (%) | 50 | 57 | 0.23 |
| Prior MI (%) | 10 | 7 | 0.30 |
| Dyslipidemia (%) | 30 | 30 | 0.95 |
| Reperfusion therapy (%) | 76 | 72 | 0.66 |
| Time to reperfusion therapy (%) | | | |
| ≤2 h | 53 | 61 | 0.12 |
| ≤6 h | 84 | 88 | 0.23 |
| ASA use (%) | 94 | 96 | 0.48 |
| Beta-blocker use (%) | 60 | 75 | 0.01 |
| UFH or LMWH (%) | 93 | 93 | 0.65 |
| Clopidogrel use (%) | 96 | 98 | 0.50 |
| ACEi or ARB (%) | 60 | 49 | 0.1 |
| Statin use (%) | 70 | 66 | 0.47 |
| Hb1Ac (%) | 5.8(0.6) | 5.8(0.53) | 0.80 |

BMI: body mass index; CK-MB: creatine kinase MB; MI: myocardial infarction; ASA: acetylsalicylic acid; UFH: unfractionated heparin; LMWH: Low-molecular weight heparin; HbA1c: glycosylated hemoglobin; ACEi: angiotension converting enzyme inhibitor; ARB: angiotensin receptor blocker; Creatinine Clearance calculated by Cockcroft-Gault; D1: Day 1 of myocardial infarction.

counterpart. Likewise, in the T-allele carrier group, both IL-2 at D5 and the increase in IL-2 from D1 to D5 were higher when compared to their counterpart. No difference was observed in 8-isoprostane levels between groups.

### 3.4. Endothelial function and plasma pool of NOx

In comparison with the CC genotype group, CT + TT subjects had a lower increment in plasma levels of NOx from D1 to D5 [6.5 (14) vs. 4.9(14) μmol/L, respectively; p < 0.001]. In addition, carriers of the T-allele (6.99 ± 4.7%) presented a significantly lower FMD at D30 than homozygotes for the C-allele (7.74 ± 4.6%; p = 0.001). This difference remained significant even after adjustment for gender, age, and waist circumference (p = 0.001).

### 3.5. Coronary angiography and CMRI

As shown in Table 2, carriers of the T-allele were more likely to have intracoronary thrombus and almost twice as likely to present a TIMI thrombus grade ≥3 or a pre-angioplasty TIMI flow grade of 0 or 1. Genotype groups shared similar characteristics regarding coronary artery disease severity, post-reperfusion TIMI flow and myocardial blush grade. Likewise, CMRI studies showed that groups did not differ in MI size, LV diastolic diameter or ejection fraction.

### 3.6. All-cause mortality

In the first 30 days after the index event, 22 primary endpoints were adjudicated (as shown Table 4). In this period of time, as depicted in Fig. 2, total mortality was significantly higher in the group of patients with the CT + TT genotypes when compared to their counterpart (11.2% vs. 4.2%; p = 0.03). The hazard ratio (HR) for this association was 2.85 (95% CI 1.03–6.99; p = 0.038) in an unadjusted analysis and 2.86 (95% CI of 1.02 to 8.00; p = 0.046) after adjusting for age and sex. The statistical significance of the association between the genotype and mortality was lost after adjustment for HOMA2%β in the Cox regression model (p = 0.16). Although beta-blocker was administered less frequently for patients with the CC genotype, there were no differences in functional class or hemodynamic status between groups. Also, there no significant association between mortality at 30 days and beta-blocker use.

**Table 2**
Angiographic and CMRI characteristics of study subjects.

| | TCF7L2 rs7903146 | | p |
|---|---|---|---|
| | CC | CT + TT | |
| Angiographic characteristics | | | |
| Three-vessel disease (%) | 16 | 20 | 0.32 |
| Two-vessel disease (%) | 34 | 31 | 0.40 |
| One-vessel disease (%) | 33 | 36 | 0.61 |
| Left main coronary disease (%) | 6 | 5 | 0.58 |
| Gensini score | 40(58) | 48(62) | 0.36 |
| Syntax | 8(13) | 8(14) | 0.74 |
| Post-reperfusion TIMI-flow <3 (%) | 17 | 10 | 0.61 |
| Post-reperfusion MBG <3 (%) | 32 | 42 | 0.20 |
| Intracoronary thrombus (%) | 22 | 36 | 0.03 |
| TIMI Thrombus Grade ≥3 (%) | 15 | 28 | 0.02 |
| Pre-PCI TIMI flow 0 (%) | 10 | 20 | 0.02 |
| Pre-PCI TIMI flow 0/1 (%) | 13 | 22 | 0.01 |
| CMRI | | | |
| Infarct size (g) | 12(13) | 12(12) | 0.41 |
| Infarct size (%) | 10(9) | 10.5(8) | 0.34 |
| Left ventricle diastolic diameter (mm) | 52 ± 8 | 49 ± 6 | 0.19 |
| Left ventricle ejection fraction (%) | 46 ± 15 | 46 ± 11 | 0.90 |

CMRI: cardiac magnetic resonance imaging.

## 4. Discussion

To the best of our knowledge, this is the first study to report the association between the *TCF7L2* rs7903146 polymorphism and lower insulin secretion during STEMI. This mutation also influenced the

**Table 3**
Plasma levels of lipids, glucose homeostasis, and inflammatory markers.

| | TCF7L2 rs7903146 | | p* |
|---|---|---|---|
| | CC | CT + TT | |
| Glucose homeostasis | | | |
| Insulin D1 (μIU/mL) | 21(29) | 18(23) | 0.006 |
| Insulin D5 (μIU/mL) | 12(16) | 10(10) | <0.001 |
| Delta Insulin | −8(28) | −5(20) | <0.001 |
| C-Peptide D1(ng/dL) | 5.1 ± 3.1 | 4.9 ± 3.1 | 0.97 |
| C-Peptide D5 (ng/dL) | 4.5 ± 3.0 | 4.0 ± 2.2 | <0.001 |
| Delta C-Peptide | −0.8(3.4) | −0.6(3.2) | <0.001 |
| Glycaemia D1 (mg/dL) | 115(31) | 116(33) | 0.59 |
| Glycaemia D5 (mg/dL) | 101(24) | 101(20) | 0.54 |
| Delta Glycaemia | −11(32) | −13(30) | 0.53 |
| HOMA2%S D1 (%) | 35(55) | 42(65) | 0.020 |
| HOMA2%S D5 (%) | 62(83) | 73(76) | <0.001 |
| HOMA2%S Delta | 17(80) | 23(65) | <0.001 |
| HOMA2%β D1 (%) | 112(83) | 100(94) | 0.003 |
| HOMA2%β D5 (%) | 105(82) | 98(77) | 0.002 |
| HOMA2%β Delta | −9.4(96) | −7.9(64) | <0.001 |
| Inflammatory markers | | | |
| CRP D1 (mg/L) | 0.4(0.8) | 0.6(1.2) | 0.99 |
| CRP D5 (mg/L) | 2.9(5.9) | 3.4(5.1) | <0.001 |
| Delta CRP | 2.2(5.7) | 2.3(5.2) | <0.001 |
| IL-2 D1 (pg/mL) | 0.6(2.4) | 0.6(1.5) | 0.69 |
| IL-2 D5 (pg/mL) | 5.0 ± 3 | 6.4 ± 4 | 0.04 |
| Delta IL-2 | 3.4 ± 2 | 5.0 ± 4 | 0.02 |
| 8-isoprostane D1 (pg/mL) | 34.7 ± 17 | 40.1 ± 22 | 0.32 |
| 8-isoprostane D5 (pg/mL) | 28.4 ± 13 | 26.1 ± 10 | 0.71 |
| Delta 8-isoprostane | −6.3 ± 19 | −10.6 ± 23 | 0.36 |

* *p value* calculated by ANCOVA adjusted for sex, age, waist circumference, and baseline levels where fitting.

**Table 4**
Causes of death in the first 30 days after admission.

| Case number | TCF7L2 Genotype | Day after MI | Cause |
|---|---|---|---|
| 1 | CT | 1st Day | VT/VF |
| 2 | CT | 3rd Day | Cardiogenic shock |
| 3 | CT | 2nd Day | VT/VF |
| 4 | CT | 4th Day | VT/VF |
| 5 | CT | 5th Day | VT/VF |
| 6 | CT | 9th Day | Sudden Death |
| 7 | CT | 4th Day | VT/VF |
| 8 | CT | 3rd Day | VT/VF |
| 9 | CT | 5th Day | VT/VF |
| 10 | CC | 2nd Day | VT/VF |
| 11 | CT | 5th Day | Fatal recurrent MI |
| 12 | TT | 19th Day | Sudden Death |
| 13 | CT | 2nd Day | Fatal recurrent MI |
| 14 | CT | 2nd Day | VT/VF |
| 15 | CC | 1st Day | VT/VF |
| 16 | CC | 23rd Day | Fatal recurrent MI |
| 17 | CT | 1st Day | VT/VF |
| 18 | CC | 24th Day | Fatal recurrent MI |
| 19 | CC | 25th Day | Cardiogenic shock |
| 20 | TT | 2nd Day | Fatal recurrent MI |
| 21 | CT | 5th Day | Stroke |
| 22 | CT | 3rd Day | VT/VF |

VT: ventricular tachycardia; VF: ventricular fibrillation; MI: myocardial infarction.

inflammatory, thrombotic, and endothelial response to STEMI. In addition, despite the preliminary nature of the survival data, the association between genotype and 30-day mortality was dependent on HOMA2%B, suggesting that insulin secretion has an important role in this interaction.

Comprising the effector portion of the Wnt signaling pathway, *TCF7L2* has been linked to regulating β-cell survival and insulin secretion. The SNP rs7903146 of the *TCF7L2* gene has been linked to post-transcriptional impairment in insulin production [14]. Consistently, the presence of the mutant T-allele of *TCF7L2* rs7903146 was associated with insulin secretion in diabetic and non-diabetic individuals in a wide range of age, ethnicity and physical activity [15,16]. The presence of the T-allele is also associated to the longterm incidence of diabetes in obese [17] and non-obese subjects with high cardiovascular risk [18].The current study added to this body of evidence the concept that carriers of the mutant T-allele also have an impaired insulin secretion under conditions of metabolic stress.

In contrast to previous reports [10,19], we found that carriers of the T-allele had higher insulin sensitivity than the other study participants. This finding was probably due to the exclusion of T-allele carriers whose insulin sensitivity was not high enough to prevent the manifestation of diabetes, which was an exclusion criterion of the study.

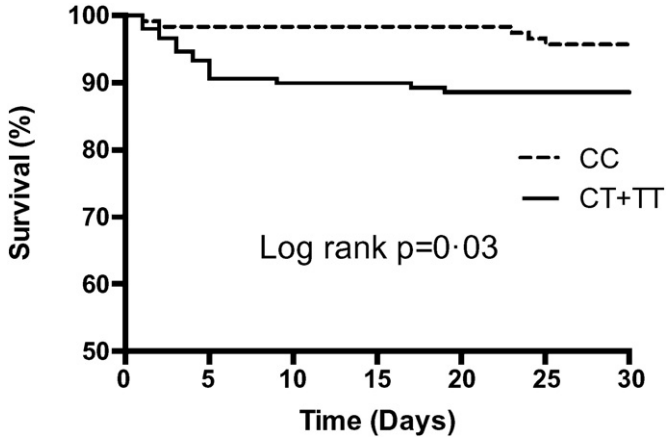


**Fig. 2.** Kaplan–Meier actuarial analysis of 30 days survival based on rs7903146 T allele presence in non-diabetic MI patients.

As remarked above, recent studies have demonstrated that, through the same signaling pathways involved in glucose lowering, insulin wields anti-inflammatory effects [2]. Indeed, insulin infusion reduces activation of nuclear factor κB (NFκB) and secretion of soluble intercellular adhesion molecule-1 (sICAM-1) and monocyte chemoattractant protein-1 (MCP-1) [20]. During MI, insulin infusion targeted to maintain blood glucose less than 140 mg/dL reduced plasma CRP and serum amyloid A [21]. Although we were not able to identify differences in oxidative stress as estimated by 8-isoprostane levels between genotypes, we found that patients with CC genotype have reduced values of IL-2 and CRP. Since both CRP and IL-2 are robust markers of inflammatory response during STEMI one could infer that under stressful conditions, such as MI, this polymorphism and its effect on insulin output could potentially influence the inflammatory response.

According to *in vitro* studies using endothelial cells, insulin increases NO production by activating endothelial NO synthase (eNOS) via the phosphatidylinositol 3-kinase pathway [22]. This effect has been mainly considered as the mechanism by which physiologic concentrations of insulin induce arterial vasodilation [23] and increase myocardial blood flow in a dose-dependent manner [3]. Accordingly, we observed that patients with the CC genotype, who had higher HOMA2%B, also presented higher FMD and NOx levels.

Upon assessment of coronary angiography results, we found that carriers of the mutated-allele also had a higher thrombotic burden. So far, this is the first report of the interaction between insulin and thrombus generation during acute coronary syndromes. The insulin receptor has been previously described in human platelets [24]. Furthermore, insulin has been reported to have an anti-aggregating effect *in vitro* and *in vivo* [4,25,26]. From a mechanistic point of view, higher physiologic concentrations of insulin stimulate NO production and increases platelet content of cGMP and cAMP [4]. In parallel, insulin may also reduce plasma concentration of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), thus both mitigating thrombogenesis and favoring thrombolysis [5].

From a clinical perspective, our study suggests that the presence of the T-allele may favor an enhanced mortality during the first 30 days after STEMI. This association looses statistical significance when adjusted by HOMA2%B, suggesting a mediating role for insulin secretion. These are preliminary findings that are nonetheless consistent with the above-mentioned effects on the inflammatory response, endothelial function, and thrombogenesis. Granted there is an apparent contradiction between our findings and clinical trials testing exogenous insulin administration in patients with MI [27,28]. However, marked differences exist between endogenous and exogenous insulin supply. For instance, exogenous insulin administration is non-pulsatile and reaches a considerably higher concentration (up to 40 times higher) [29]. In fact, exogenous non-pulsatile infusions or supraphysiological insulin doses favor insulin resistance [30], gluconeogenesis [31], endothelial dysfunction [32], and higher systemic inflammatory activity [33].

Timing till insulin infusion after myocardial ischemia could also justify the difference between this study and exogenous insulin infusion trials during MI. Although late insulin infusion did not reduce cardiovascular events in STEMI patients, this benefit was seen when insulin infusion was started early after STEMI [34]. In our study, it is reasonable to suggest that the time between stimuli and insulin response was minimal and distinct between carriers and non-carriers of the T-allele because there is no insulin administration

Some limitations of our study should be acknowledged. Firstly, we did not account for possible additive effects of other known *TCF7L2* polymorphisms or other T2D risk alleles unrelated to the Wnt pathway. For example, a possible crosstalk between the Wnt and forkhead box O (FOXO) pathways could attenuate inflammatory response [14] and favor eNOS activity [35]. Moreover, the *TCF7L2* gene is also expressed in non-pancreatic cells [36] and may exert distinct effects that impact recurrence of events in a manner that is unrelated to insulin secretion

*per se*. Thus, it is possible that a reduced insulin secretion is not the only mechanism that would benefit STEMI patients. Nonetheless, the effect on insulin secretion cannot be neglected because the presence of the T-allele had marked impact in insulin secretory function and the adjustment for insulin secretion nullified the association between the polymorphism and mortality after MI.

We found imbalance favoring a slightly higher frequency of T-allele carriers (56%) as compared with subjects without coronary artery disease (51%) [17], and to those with chronic stable disease (53%) [37]. This is in line with the higher severity of coronary artery disease in non-diabetic T-allele carriers [37] and higher incidence of stroke in individuals with TT-genotype [18]. We also found a lower frequency of TT-genotype (8.5%) in our cohort, which may represent a survival bias since patients who died before hospitalization were not assessed. These assumptions, however, requires further investigation.

In conclusion, the *TCF7L2* rs7903146 is associated with decreased insulin secretion, increased inflammatory response, thrombotic burden, and endothelial dysfunction in STEMI patients. By comparing distinct insulin secretory phenotypes, this finding supports the concept that carriers of *TCF7L2* rs7903146 C allele have a favorable impact on the adaptive response and clinical outcome of STEMI patients.

## Funding

Brazilian National Research Council (CNPq) and Foundation for Research Support of the State of São Paulo (FAPESP).

## Duality of interests

No potential conflict of interest relevant to this article is reported.

## Transparency Document

The Transparency document associated with this article can be found, in online version.

## Acknowledgment

Filipe A. Moura was supported by a doctoral grant (number 151900/2011-6) from the Brazilian National Research Council (CNPq). Dr. Figueiredo was supported by a post-doctoral fellowship (grant number 2012/18044-1) from the Foundation for Research Support of the State of São Paulo (FAPESP). L. S. F. Carvalho was supported by a doctoral grant from the Coordination for the Improvement of Higher Education Personnel (grant number 01P-4353/2015) (CAPES). Prof. Sposito was supported by a fellowship grant of productivity in research from CNPq (grant number 300313/2007-1).

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